ACS SyntheticBiology

In This Issue

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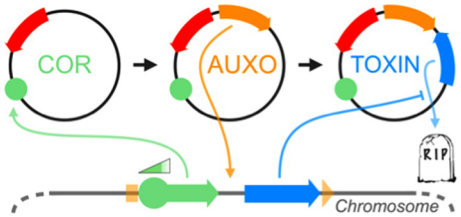
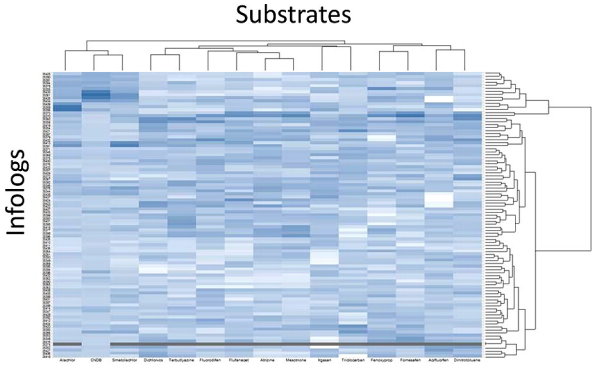
GENEGUARD: A MODULAR PLASMID SYSTEM FOR BIOSAFETY



The release of engineered microbes into the environment raises concerns of unchecked cellular proliferation and unwanted spread of synthetic genes. Here, Wright *et al.* (DOI: 10.1021/sb500234s) describe the development and implementation of "GeneGuard", a novel vector system designed to reduce the likelihood of transfer of synthetic DNA to microbes other than the intended hosts.

GeneGuard uses redesigned modular plasmids as vectors for synthetic biology applications that require release in environments where horizontal gene transfer to native organisms is a concern. The authors illustrate the efficacy of GeneGuard with heavy-metal biosensor applications and demonstrate that it works to reduce gene transfer not only into eubacteria, but also into soil bacteria. The modular GeneGuard system could be a valuable tool for taking synthetic biology out of the lab and implementing it in real-world scenarios.

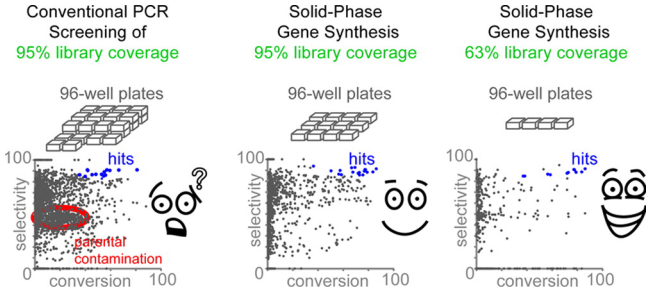
MAPPING OF AMINO ACID SUBSTITUTIONS CONFERRING HERBICIDE RESISTANCE



Over the past two decades, directed evolution has revolutionized the field of protein engineering with far reaching implications from structure–function correlation studies to commercialization of engineered biological systems. In parallel, there has been an advent of massive genomic sequencing efforts, big data analysis and efficient gene synthesis allowing for a direct synthetic biology link between virtual information and physical genes. The development of statistical tools for the efficient mapping of the mega-dimensional sequence-function landscape of proteins is essential. In this study, Govindarajan *et al.* (DOI: 10.1021/sb500242x) outline a method for integrating systematic variance, multivariate data analysis and gene synthesis to efficiently search protein engineering space.

The authors use the glutathione synthase as a case study for how to navigate toward multiple different functional activities in parallel. The method described is generic and directly applicable to engineering any DNA encoded properties.

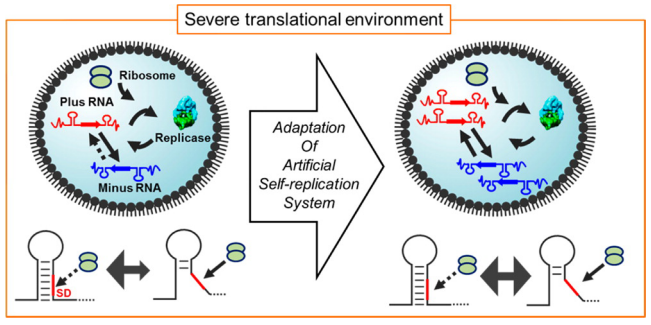
SPEEDING UP DIRECTED EVOLUTION



While directed evolution is widely used in basic and applied biological research and bioengineering, more efficient and economic methods are necessary for directed evolution at the protein, metabolic and genomic levels. Techniques such as PCR and DNA shuffling continue to be used, but semirational approaches based on saturation mutagenesis allow researchers to focus on important functional sites of these proteins, metabolic pathways and genomes. Here, Hoebenreich *et al.* (DOI: 10.1021/sb5002399) discuss the economic/operational aspects of saturation mutagenesis experiments while addressing whether large protein libraries are best prepared by PCR or by synthetic approaches.

The authors compare the complete directed evolution process between three different homemade PCR-based (using MegaPrimer) and commercial solid-phase gene synthesis (Sloning) libraries based on saturation mutagenesis. They show that solid-phase gene synthesis is a superior alternative to PCR-based methods for creating mutant libraries, and that considerably smaller library sizes are often satisfactory. This work promises to be a useful reference for the directed evolution community.

ADAPTIVE EVOLUTION OF AN ARTIFICIAL RNA GENOME TO A REDUCED RIBOSOME ENVIRONMENT



The synthesis of an artificial reaction system with the ability to evolve and adapt to its environment is a major challenge in the field of *in vitro* synthetic biology. Here, Mizuuchi *et al.* (DOI:

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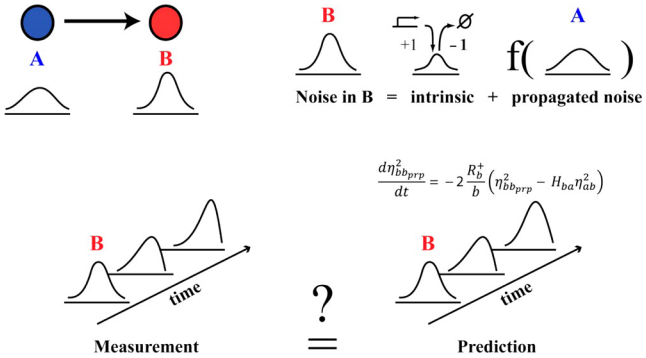
10.1021/sb5000884) describe an example of adaptive evolution *in vitro* using an artificially reconstituted RNA genome replication (TcRR) system. While this system has been previously reported to simulate Darwinian evolution, this study uses a "severe translation environment" with a reduced ribosome concentration to confer selective pressure and to test the TcRR system's ability to simulate adaptive evolution.

The authors found that the genomic RNA adaptively evolved to the environment by changing its RNA structure. The replication efficiency of the system was comparable to that in the original environment. Thus, the mutant genome compensated for the reduced ribosome concentration, representing a step toward enabling adaptive evolution *in vitro*.

USING DYNAMIC NOISE PROPAGATION TO INFER CAUSAL REGULATORY RELATIONSHIPS



The fast emerging technologies measuring gene expression in single-cells provide a rich picture of variability across a population. It is clear that this variability, or "noise", is information rich. However, methods that rigorously exploit these data to provide insight into the underlying networks, especially under nonsteady state conditions, are still lacking. Now, Lipinski-Kruszka *et al.* (DOI: 10.1021/sb5000059) describe how such data can be analyzed and utilized to identify gene-regulatory motifs.

To do this, the authors derived mathematical equations that allowed them to predict how a population's variability is expected to evolve over time under different regulatory relationships. They tested each of these relationships by comparing the noise trajectories generated by different models with the trajectories measured experimentally using flow cytometry. Applicability of the method was demonstrated using *in silico* data obtained from stochastic simulations, as well as with *in vivo* data from synthetic circuits constructed and expressed in *S. cerevisiase*. In all cases, dynamic noise information was seen to provide discriminatory power between different regulatory relationships. Thus, this method represents an important advance in the effort to exploit noise information.

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